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DETERMINATION OF LETHAL DOSE AND EFFECT OF GAMMA RAY ON GERMINATION PERCENTAGE AND SEEDLING PARAMETERS IN ADT (R) 47 RICE

[a*]Rajarajan, D., [b]Saraswathi, R. & [c]Sassikumar, D.
[a, b] Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India.
[c] Plant Breeding and Genetics Unit, Tamil Nadu Rice Research Institute, Aduthurai - 612101, Tamil Nadu Agricultural University, Tamil Nadu, India.
[*]Corresponding author email: rajarajandeva@gmail.com

ABSTRACT
An experiment was conducted to estimate the lethal dose of the physical mutagen gamma ray in rice cultivar ADT (R) 47. Genetically pure seeds were treated with different doses of gamma rays *viz.*, 150Gy, 200Gy, 250Gy, 300Gy and 350Gy. Untreated seeds soaked in distilled water were used as check for comparison. The LD50 values were observed based on growth reduction of seedlings after gamma ray treatment. The LD50 dose for gamma ray under *in vitro* and *in vivo* condition was fixed at 229Gy and 235Gy based on probit analysis. As the doses of applied gamma ray increased, there was a decrease in germination, survival rate of seedlings, root length, shoot length, seedling height, vigour index under *in vitro* conditions and emergence and survival under field (*in vivo*) conditions in M1 generation as compared to the control. The survival % of seedlings was higher at higher doses of gamma ray under *in vivo* conditions compared to *in vitro* conditions.

KEY WORDS: Rice, Gamma rays, Lethal Dose 50 (LD 50).

INTRODUCTION

Induced mutation by use of either physical or chemical mutagen is one way of creating variation in crop plants. The physical mutagens comprise of ionising radiation *viz.*, particulate (alpha rays, beta rays, fast neutrons and thermal neutrons) and non-particulate also called as electromagnetic radiation (X rays and gamma rays). The mutagenic action of X-ray was discovered by Muller (1927) in *Drosophila* and of gamma rays and X-rays in 1928 by Stadler in barley and maize, *Datura stramonium* (Gager and Blakeslee, 1927) and *Nicotiana* (Goodspeed, 1929), which opened up a new field of science in genetics. For inducing mutation various radiations can be used. According to Kovacs and Keresztesa (2002) gamma rays are considered as the most penetrating in comparison to other radiation such as alpha and beta rays. Many physical mutagens have been used for obtaining superior mutants in rice for different traits (Singh *et al.*, 1998). Genetic variability induced in rice through gamma rays for selecting new genotypes with improved rice quality and high yield potentials have been documented (Rutger, 1983). The identification of most effective mutagenic treatment and efficient mutagens is very essential to recover a high frequency and spectrum of useful mutations (Smith, 1972; Solanki *et al.*, 1994; Kumar, 1998).

MATERIALS & METHODS

In mutation breeding, to avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50} which is the safe dose at which half of the planting materials survive. The sensitivity of mutagens which enables the breeders to improve the genotype can also be judged with the help of LD_{50} (Cheema and Atta, 2003; Mensah *et al.*, 2005) and pollen fertility (Jayabalan and Rao, 1987). The present investigation aims at fixation of LD_{50} dose for gamma rays in rice cultivar ADT (R) 47. The seeds of rice variety namely ADT (R) 47 for the induction of mutation treatment was obtained from Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu Agricultural University (TNAU), Coimbatore. Well filled, healthy and uniform sized seeds handpicked from the seed lot and equilibrated to the moisture content of 12 per cent were packed in butter paper covers (200 seeds per treatment). The gamma chamber (GC 1200) installed by Board of Radiation and Isotope Technology (BRIT), Govt. of India, Mumbai at Centre for Plant Breeding and Genetics, TNAU, Coimbatore where, ^{60}Cobalt serves as source of gamma rays was used for treatment. Seeds were placed in the Gamma chamber and exposed to gamma irradiation of eight doses *viz.*, 150Gy, 200Gy, 250Gy, 300Gy and 350Gy for appropriate time for each dose based on the half life of the source. Non-irradiated dry seeds were taken as control. The irradiated seeds were sown the next day. The treated seeds were placed in roll paper towels for germination test under *in vitro* condition with two replications. Another set of treatment was carried out and the seeds were sown in raised beds in the field (*in vivo*) immediately after the treatment along with the control seeds. Under *in vitro* condition, germination %, survival % (14 DAT), shoot length, root length and vigour index were observed. Germination % and survival % were observed three days and 14 days after treatment (DAT) for both *in vitro* and *in vivo* conditions. Shoot length and root length of the seedling of different treatments were also measured on 14 DAT. Vigour index was calculated by multiplying

germination % and total seedling length (cm). Lethal Dose experiment was organized based on a completely randomized block design with two replications and the random block included six levels of gamma ray treatment (including control). Least significant difference (LSD) test at P-values less than 0.01 was used to investigate the differences in observed averages of all tested parameters between treated and non-treated plants. Probit analysis (Finney 1971, 1978) was carried out to determine the lethal dose (LD_{50}) of gamma ray under *in vitro* and *in vivo* conditions.

RESULTS & DISCUSSION

Lethal dose, the percentage of test material that are killed by a specific dosage of chemicals or radiation in which half will die, is the optimum dose that causes high frequency of favourable mutations with minimum damage to the plant. Doses lower than LD_{50} favour plant's recovery after treatment, while the use of higher doses increases the probability to induce mutations either in positive or in negative direction. Before the start of an experiment in induced mutations, fixation of LD_{50} is important. Moreover, this value varies with biological materials, nature of treatment and subsequent environmental conditions (Singh, 1994; Babaei *et al*., 2010). In the present investigation, the seeds of ADT (R) 47 were treated with gamma rays (150Gy to 500Gy with 50Gy interval). For a sample size of 200 seeds per dose in mutagen, probit analysis was done using seed germination values to determine the Lethal Dose (LD_{50}).The LD_{50} value for gamma irradiation under *in vitro* and *in vivo* condition was arrived as 229 Gy and 235 Gy respectively for the variety ADT (R) 47 (Fig.1a and 1b). In rice, to allow 60% survival of seedlings the effective dosage of gamma rays generally ranged from 150 to 300Gy (Rutger,1992). Cheema and Atta (2003) used three Basmati rice varieties to examine varietal differences in radio-sensitivity to gamma irradiation. They found that LD_{50} values for seed fertility were 238, 232 and 223Gy for Basmati 37, Basmati Pak and Super Basmati, respectively. Optimum dose and LD_{50} for survival of seedlings in landrace "Tarom mahalli" was determined to be 230 Gy (Hallajian *et al.,* 2014).

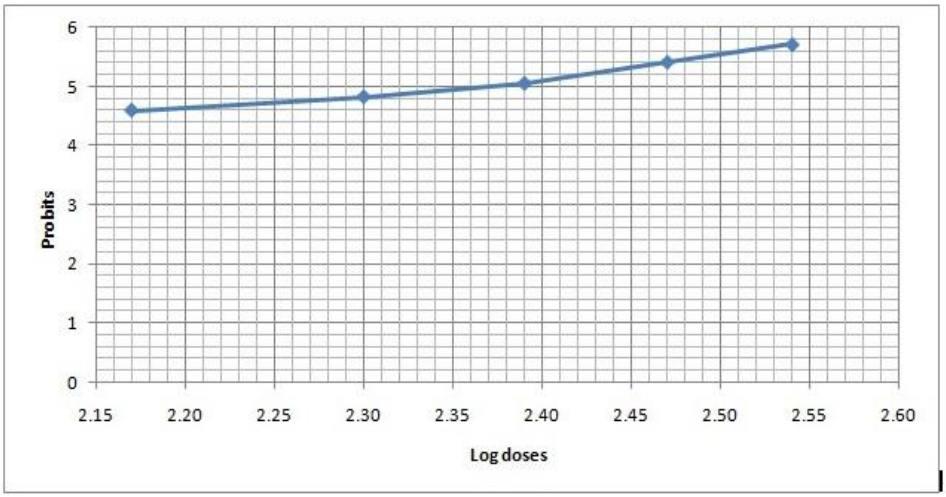

FIGURE 1a: Calculation of LD50 of gamma irradiation in ADT (R) 47 rice under *in vitro* condition

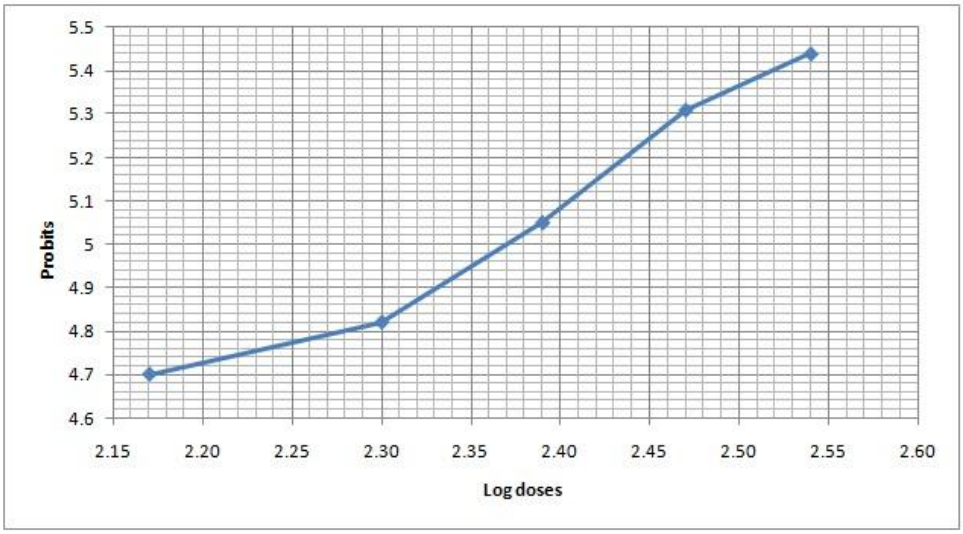

FIGURE 1b. Calculation of LD50 of gamma irradiation in ADT (R) 47 rice under *in vivo* condition

TABLE 1. Germination, survival reduction percentage and seedling parameters following gamma mutagenesis under *in vitro* condition in ADT (R) 47 rice

Treatment	Germination (%) (GP)	Survival % (reduction) in 14 DAT (SR)	Shoot length (cm)(SL)	Root length (cm) (RL)	Total seedling length (cm) (TSL)	Vigour Index (VI)
Control	93.0a	91.0a	14.1a	14.5a	28.6a	2660.1a
150 Gy	64.5b	60.0b	11.4b	12.9b	24.3b	1570.9ab
200 Gy	57.0c	51.5c	10.7c	12.4bc	23.1c	1316.9b
250 Gy	48.5d	43.5d	8.9d	12.1bc	21.1d	1023.8a
300 Gy	37.5e	31.0e	7.8e	11.7c	19.5e	733.5ab
350 Gy	26.5f	21.5f	7.3e	10.2d	17.6f	466.6b
Mean	54.5	49.7	10.0	12.3	22.3	1295.3
SE(d)	2.7	2.0	0.2	0.3	0.3	473.2
CV %	4.9	4.1	2.2	2.9	1.3	36.5

The values are mean of two replicates under *in vitro* condition.

For gamma rays under, in *in vitro* condition, seedling mortality per cent showed variation over the treated population at each dose (Table 1) which was 78.5 per cent at 350Gy, 69.0 per cent at 300Gy, 56.5 per cent at 250Gy, 48.5 per cent at 200Gy and 40.0 per cent at 150Gy. Similar trend in variation was observed under *in vivo* condition, with a mortality percent of 68.7 at 350Gy, 64.5 at 300Gy, 54.3 at 250Gy, 46.5 at 200Gy and 41.0 at 150Gy (Table 2). Under *in vitro* and *in vivo* condition, the percent reduction in germination over control/parent and doses of gamma rays followed a linear trend. The maximum values of percent reduction in germination were recorded at 350Gy dose. Such a dose dependent decrease in germination was also reported by Talebi *et al*. (2012); Tabasum *et al*. (2011). The reduction in germination was reflected on the survival % of seedlings on 14th day which also exhibited the same trend. The maximum values of percent reduction in survival over parent were at 350Gy dose. Reduced growth of seedlings after mutagenic treatments has been explained on the basis of auxin destruction, changes in ascorbic acid content and physiological injury and biochemical disturbances (Yusuf and Nair, 1974).

The observation on shoot length, root length, total seedling length and vigour index on gamma ray induced mutants showed a significant effect on all the traits as compared to the control (Table 1). The maximum shoot length (11.4cm), root length (12.9cm) and total seedling length (24.3cm) was observed at minimum dose of 150Gy. The next higher values for these parameters were observed at 200Gy. The least values were noticed at 350Gy. Dehpour *et al*. (2011) observed that the maximum decrease in shoot length was observed when rice genotypes were exposed by gamma ray dose higher than 200 Gy. Higher reduction of root length was observed at higher dose of gamma rays of 350 Gy. These results are in agreement with Ashraf *et al*. (2003); Tabasum *et al*. (2011); Kadhimi *et al*. (2016). Vigour index also followed the same pattern exhibiting the maximum value (1570.9) at 150Gy and the minimum value (466.6) at 350Gy (Fig. 2).

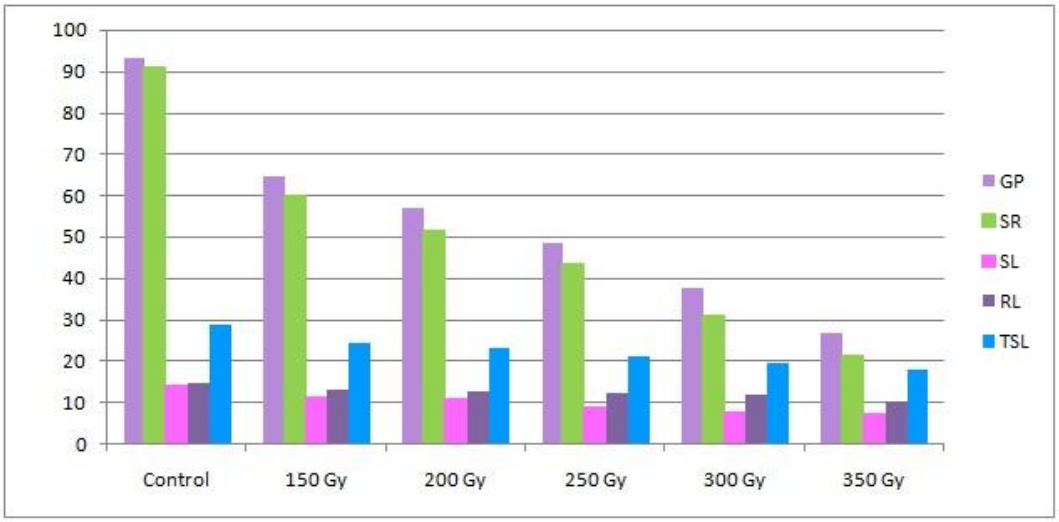

FIGURE 2: Effect of gamma irradiation dose on germination % and seedling parameters ADT (R) 47 rice under *in vitro* condition

The percent reduction in germination over control increased with the dose of the mutagen and it ranged from 36.0 (150Gy) to 62.2 (350Gy). The reduction in survival % of the seedlings at 14 days after treatment also exhibited the same trend (Table 2) and it ranged from 37.7 (150Gy) to 66.9 (350Gy). A comparison of both the parameters at different doses revealed that the survival reduction per cent was more pronounced at higher doses of 300Gy (62.5) and 350Gy (66.9). Under both conditions, gamma rays imparted a significant effect on shoot length, root length,

total seedling length and vigour index in the present investigation. The highest dose of 350Gy caused severe reduction in all the parameters, while the lowest dose (150Gy) showed the highest expression for these characters. The greater sensitivity at higher doses of mutagens has been attributed to various factors such as changes in metabolic activity of cells and disturbances of balance between promoters and inhibitors of growth regulators (Meherchandani, 1975). In Radha 4 rice variety, Basi *et al*. (2006) reported that 35kR dose of gamma rays was the most potent dose inducing cytogenetic aberrations in meiotic pollen mother cell of rice compared to other dose spectrum.

TABLE 2. Reduction in germination and survival over control percentage after gamma irradiation of ADT (R) 47 rice under *in vivo* condition

Treatment	Germination %	% reduction over control	Survival % (14 DAT)	% reduction over control
Control	95.3	0	94.7	0
150 Gy	61.0	36.0	59.0	37.7
200 Gy	55.7	41.6	53.5	43.5
250 Gy	48.3	49.3	45.7	51.8
300 Gy	41.5	56.5	35.5	62.5
350 Gy	36.0	62.2	31.3	66.9

CONCLUSION

The present study revealed that the lethal dose for ADT (R) 47 rice to be 229 Gy dose of gamma ray under laboratory conditions through assessment of traits such as seed germination, survival rate of seedlings, shoot length, root length and vigour index in M_1 generation. Under field conditions, based on germination %, survival of seedlings, the dose was fixed at 235 Gy through probit analysis. LD_{50} values may be utilized for launching a successful mutation breeding program and this indicates the potential of gamma irradiation in rice which could be of immense significance for the assessment of irradiation treatment for efficiently obtaining desirable mutants without disturbing the major part of the genotypic or phenotypic architecture of the crop plants.

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REFERENCES

Ashraf, M., Cheema, A. A., Rashid, M and Qumar, Z. U. (2003) Effect of gamma rays on M_1 generation in basmati rice. *Pak. J. Bot.* 35(5): 791- 795.

Babaei, A., Nematzadeh, G.A., Avagyan, V., Hamidreza, S and Petrodi, H. (2010) Radio sensitivity studies of morpho-physiological characteristics in some Iranian rice varieties (*Oryza sativa* L.) in M_1 generation. *Afr. J. Agrl. Res.* 5(16): 2124- 2130.

Basi, S., Subedi, L. P., KC, G. B and Adhikari, N. R. (2006) Cytogenetic effects of gamma rays on Indica rice Radha-4. J. *Inst. Agric. Anim. Sci.* 27: 25-36.

Cheema, A. A. and Atta, B. M. (2003) Radio sensitivity studies in basmati rice. *Pak. J. Bot.* 35(2): 197- 207.

Dehpour, A. A., Gholampour, M., Rahdary, P., Talubaghi, M. R. J and Hamdi, S. M. M. (2011) Effect of gamma irradiation and salt stress on germination, callus, protein and proline in rice (*Oryza sativa* L.). *Iran. J. Plant Physiol.* 1(4): 251-256.

Finney, D. J. (1971) Probit analysis (3rd edition) Cambridge University Press, Cambridge, UK.

Finney, D. J. (1978) Statistical method in biological assay. Charles Griffin and Co.edn

Gager, C. S and Blakeslee, A. F. (1927) Chromosome and gene mutations in *Datura* following exposure to radium rays. Proceedings of the National Academy of Sciences of the USA, 13: 75- 79.

Goodspeed, T. H. (1929) The effects of X-rays and radium on species of the genus *Nicotiana. J. Hered.* 20: 243- 359.

Hallajian, M. T., Ebadi, A. A., Mohammadi, M., Muminjanov, H., Jamali, S. S and Aghamirzaei, M. (2014) Integration of mutation and conventional breeding approaches to develop new superior drought-tolerant plants in Rice (*Oryza sativa*. L). *Annual Research and Review in Biology*, 4(7): 1173-1186.

Jayabalan, N and Rao, G. R. (1987) Effect of physical and chemical mutagens on chiasma frequency in *Lycopersicon esculentum* Mill. *Caryologia*, 40: 115-121.

Kadhimi, A.A., ALhasnawi, A N., Isahak, A., Ashraf, M.F., Mohamad, A., Yusoff, W.M.W and Zain, C.R.C. M. (2016) Gamma radio sensitivity study on MRQ74 and MR269, two elite rice varieties (*Oryza sativa* L.) *Journal of Life Scienc*. 13(2): 85-91.

Kovacs, E. and Keresztesa, A. (2002) Effect of gamma and UV-B/C radiation on plant cell. *Micron*, 33: 199-210

Kumar, H.D.M. (1998) Frequency and spectrum of chlorophyll mutations induced by gamma- rays in two rice varieties. *Karnataka Journal of Agricultural Sciences,* 11**:** 637- 640.

Meherchandani, M. (1975) Effect of gamma radiation on the dormant seeds of *Avena sativa* L. *Rad. Bot.* Pp. 439 – 455.

Mensah, J.K., Akomeah, P.A. and Ekpekurede, E.O. (2005) Gamma irradiation induced variation of yield parameters in cowpea (*Vigna unguiculata* L.) Walp. *Global Journal of Pure Applied Sciences,* 11(3): 327-330.

Rutger, J.N. (1983) Applications of induced and spontaneous mutation in rice breeding and genetics. *Advances in Agronomy,* 36: 383-413

Rutger, J.N. (1992) Impact of mutation breeding in rice - A review. *Mutat. Breed. Rev.*8: 1-24.

Singh, B. D. (1994) Mutations in crop improvement. In**:** Plant breeding Kalyani Publ. Ludhiana. Pp. 424-450.

Singh, S., Richharia, A.K. and Joshi, A.K. (1998) An assessment of gamma ray induced mutations in rice (*Oryza sativa* L.). *Indian J. Genet.* 58(4): 455- 463.

Smith, H. H. (1972) Comparative genetic effects of different physical mutagens in higher plants. In: joint FAO/IAEA. Division of Atomic Energy in Food and Agriculture, ed. Induced Mutations and Plant Breeding Improvement IAEA. Vienna, 75-93.

Solanki, I. S. and Sharma, B. **(**1994) Mutagenic effectiveness and efficiency of gamma rays, ethylene imine and N-nitroso-N-ethyl urea in macrosperma lentil (*Lens culinaris* Medik.). *Indian J. Genet.* 54(1): 72-76.

Tabasum, A.Q.S.A., Cheema, A.A., Hameed, A., Rashid, M and Ashraf, M. (2011) Radio sensitivity of rice genotypes to gamma radiations based on seedling traits and physiological indices. *Pak. J. Bot.* 43(2): 1211-1222.

Talebi, A.B., Talebi, A. B and Jafarpour, M. (2012) Identify the lethal dose of EMS and gamma radiation mutagenesis in Rice MR219.In: 2nd International Conference on Environment Science and Biotechnology, IPCBEE, Singapore, 48 (5): 22-26.

Yusuf, K.K. & Nair, P.M. (1974) Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes. *Radiation Bot.* 14: 251-256.